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GRANT#: N00014-91-J-4082 **FINAL REPORT**

PRINCIPAL INVESTIGATOR: F. Gerald Plumley

INSTITUTION: Institute of Marine Science
University of Alaska Fairbanks

GRANT TITLE: Marine Diatom Plasmids and their Biotechnological Applications

REPORTING PERIOD: Through 2-27-92

OBJECTIVE:

The long-range objective of the proposed research is to achieve transformation of marine diatoms. The more immediate objectives pertain to the characterization of two small plasmids we have discovered in a marine diatom, Cylindrotheca fusi. .mis, a species which comprises 50% of the biofouling community on steel structures in marine environments. Specific questions being addressed are:

1) What are the structural, functional and regulatory properties of the two diatom plasmids?
2) Are there sequences on these plasmids which will facilitate construction of diatom shuttle-cloning vectors?
3) What are the necessary conditions for achieving diatom transformation?

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

ACCOMPLISHMENTS (three months support):

We have isolated two small plasmids (of about 4.2 and 4.0 kbp) from the marine diatom Cylindrotheca. The 4.4 kbp plasmid has three Eco R1 sites and these have been exploited for cloning into pBluescript vectors. We have also cloned at least one Eco R1 fragment from the 3.6 kbp plasmid and are screening our library for the remaining fragment(s).

We have sequenced approximately 3000 bp of the 4.2 kbp plasmid. A search of the Gene Bank library has lead to the conclusion that this plasmid is homologous to the Tn21-type transposable elements. The element carries an open reading frame encoding a DNA invertase gene. Sequence comparisons of regions upstream and downstream of the invertase gene indicate that the diatom plasmid is most similar to the *Staphylococcus aureus* transposon Tn552. Southern blots using the entire diatom plasmid as probe demonstrate that plasmid sequences are not present elsewhere in the diatom genome. Based on the highly prokaryotic nature (i.e., codon usage bias, promoter sequences, etc.) of the invertase gene we have sequenced, we have tentatively ascribed it to be localized in the chloroplast. Cloned fragments of the invertase gene expressed under the control of its own promoter yield a 20 kd

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protein (the size of the bacterial protein) in transgenic *E. coli*.

We have also determined that "all" diatoms species harbor sequences common to the plasmids of Cylindrotheca. More specifically, each plasmid carries the site-specific invertase gene. Surprisingly, we also find that similar sized plasmids are present in other unicellular algae such as Chlamydomonas and that these plasmids also carry sequences which hybridize to the site-specific invertase gene.

PUBLICATIONS AND REPORTS (three months):

Ribbens, Peter. 1991. Isolation and characterization of a novel plasmid from a marine diatom.
(This was an abstract graduate student Peter Ribbens to Sea Grant for consideration in their National Student Research Competition. The abstract won first place in the Biotechnology Division.)

Ribbens, P. and F.G. Plumley. 1991. Multiple Plasmids in a Marine Diatom, Cylindrotheca fusiformis. XI North American Diatom Symposium. Clemson University.

Ribbens, P. and F.G. Plumley. Bacterial-like transposable elements in marine and fresh water eukaryotic algae. FEBS Lett. (in prep).

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